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Sperm Count, Morphology and Fluorescent Body Frequency in Autopsy Service Workers Exposed to Formaldehyde

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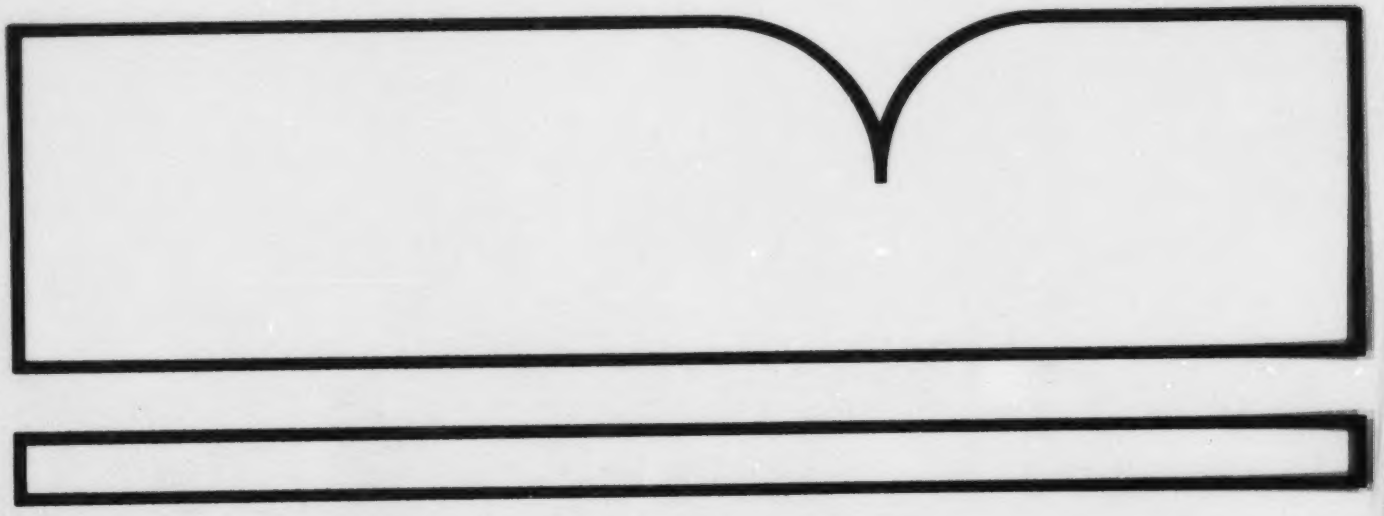

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16. ABSTRACT

The ability of a battery of genetic monitoring tests to detect occupational formaldehyde exposure in a population of a hospital autopsy service workers was investigated. Eleven exposed individuals and 11 matched controls were evaluated for sperm count, abnormal sperm morphology and 2 F-body frequency. Subjects were matched for sex, age and customary use of alcohol, tobacco and marijuana. Additional information was collected on health, medications and other exposures to toxins. Ten subjects were employed for 4.3 months (range 1 to 11 months) prior to the first sample and one was employed for several years. Formaldehyde exposures were episodic but with a time weighed average between 0.61 and 1.32 pm (weekly exposure range 3 to 40 ppm. hrs). Exposed and control subjects were sampled 3 times at 2 to 3 month intervals. Sperm morphology was also evaluated in $B_6C_3F_1$ mice after 5 daily oral doses of 100 mg/kg formalin. No increase in abnormal morphology was detected in the treated animals. In humans, no statistically significant differences were observed between the exposed and control groups for the observed variables. Reduced sperm count correlated with increased abnormal morphology and 2-F body frequency in the exposed group but not in the control group. Evaluation of the impact of incidental exposures suggests a reduced count with marijuana use and increased abnormal morphology with medications used by controls.

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Sperm count, morphology and fluorescent body frequency in autopsy service workers exposed to formaldehyde

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Summary

A battery of monitoring tests that could indicate genetic damage was used to investigate occupational formaldehyde exposure in a population of a hospital autopsy service workers. 11 exposed individuals and 11 matched controls were evaluated for sperm count, abnormal sperm morphology and 2F-body frequency. Subjects were matched for sex, age and customary use of alcohol, tobacco and marijuana. Additional information was collected on health, medications and other exposures to toxins. 10 subjects were employed for 4.3 months (range 1–11 months) prior to the first sample and 1 was employed for several years. Formaldehyde exposures were episodic but with a time weighed average between 0.61 and 1.32 ppm (weekly exposure range 3–40 ppm · h). Exposed and control subjects were sampled 3 times at 2–3 month intervals. Sperm morphology was also evaluated in $B_6C_3F_1$ mice after 5 daily oral doses of 100 mg/kg formalin. No increase in abnormal morphology was detected in the treated animals. In humans, no statistically significant differences were observed between the exposed and control groups for the observed variables. Reduced sperm count correlated with increased abnormal morphology and 2F-body frequency in the exposed group but not in the control group. Evaluation of the impact of incidental exposures suggests a reduced count with marijuana use and increased abnormal morphology with medications used by controls. No effects on sperm were seen from formaldehyde or its metabolites in this population after occupational exposure, nor in mice following a high acute exposure. It is possible that minor effects might have occurred. The lack of an effect in this study may be due to a lack of statistical power to detect effects at this exposure level.

Formaldehyde is a widely used industrial chemical. The general public is exposed to low to moderate levels of formaldehyde in many materials including textiles, and building products such as foam insulation and particle board. In addition, occupational exposures to formaldehyde and its derivatives may involve 1.4–1.75 million workers in the United States (IARC, 1983). Formaldehyde has been shown to produce DNA damage and mutation in a variety of biological systems, including bacteria, fungi, Drosophila larvae, cultured mammalian cells and rodents. Dominant lethal tests in the mouse with formaldehyde alone (Fontignie-Houbrechts, 1981) and formaldehyde

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with hydrogen peroxide as well as adenine formaldehyde bis-nucleotides (Fontignie-Houbrechts et al., 1982) detected early pre- and post-implantation loss, although no effects were seen in spermatogonial metaphase chromosomes. The genetic effects of formaldehyde have been recently reviewed (IARC, 1983; Griesemer et al., 1982). Formaldehyde has been shown to be carcinogenic, producing dose-related increases in squamous cell carcinomas of the nasal turbinates after chronic inhalation exposure in Fisher 344 (Anon, 1982) and Sprague–Dawley (Albert et al., 1982) rats. The preponderance of evidence suggests that human exposure carries at least a potential risk of adverse health effects from genetic damage.

Human genetic monitoring is an epidemiological approach in which observation of the immediate effects of mutagen exposure are substituted for observation of adverse health outcomes. This eliminates the time interval which must occur between exposure and outcome. Furthermore, the frequency of these effects is much greater than the frequency of disease. As a result, the presence of a hazardous exposure can be detected after much shorter periods of time and with much smaller populations using genetic monitoring techniques than by looking for elevated incidence of adverse health outcomes such as cancer or reproductive abnormalities (Ward, in press).

In this study, we evaluated a male population of pathologists and their co-workers employed full or part-time on the Autopsy Service of the University of Texas Medical Branch, Galveston, TX, to determine whether their formaldehyde exposure could be detected by tests in sperm. Results are presented for 3 variables: count, morphology and proportion containing zero, one or two fluorescent bodies (2F-body frequency which is thought to measure chromosome non-disjuction). For review of these variables, see Wyrobek et al. (1983a). Sperm morphology analysis conducted in formaldehyde-treated mice is also reported. A detailed description of the study design for our evaluation of genotoxic effects of formaldehyde in humans and mice has been published (Ward et al., 1983).

Methods

Population recruitment, evaluation and selection. Subjects for the exposed group were recruited from the staff of the Autopsy Service and for the non-exposed group from the staff and students at the Medical Branch. Respondents were asked to complete a questionnaire which inquired about age, sex, occupation, potential sources of occupational exposure to toxic substances, non-occupational exposure to toxic substances, customary use of tobacco, alcohol and recreational drugs, history of major illnesses, recent history of minor illnesses and current and past use of medications. Each respondent in the exposed group was matched with a respondent in the non-exposed group. Criteria for matching were (in order of importance) sex, age (to within 5 years), and customary use of tobacco, alcohol and recreational drugs (marijuana). Health, medications and incidental exposures to solvents, pesticides or diagnostic radiation were not used as a basis for matching but were considered in selecting subjects for study and in evaluating results. Information on coffee consumption was collected following the selection of the study groups and was considered in evaluating results. Most subjects were selected at the beginning of the study. However, 2 pathology residents rotating through the autopsy service and a newly hired technician were recruited and matched with controls selected from the pool of original respondents. The study population consisted of 5 residents, 5 technicians and a pathologist. 4 of the residents served for 6 months on the service beginning 2 months before the first sample collection and ending 2 months before the third sample collection. The 5th resident served for 6 months on the service ending one month prior to the collection of the first sample. The newly hired technician was sampled 1, 3 and 7 months after beginning work. All other participants had worked on the service for at least 6 months prior to study including a faculty member with more than 10 years service.

Sample collection and processing. Male subjects provided 3 semen specimens at intervals of 2–3 months except where noted under Results. Samples were collected in the morning by the subject at home by masturbation into a sterile

container and delivered to the laboratory, usually within 2 h. The times of collection and delivery were noted. Sample volume was determined and general quality (e.g. color, viscosity and odor) noted. The period of prior continence was requested at the time of sample delivery. Air-dried slides were prepared as described by Kapp and Jacobson, (1980).

Sperm count. The sperm number was determined on a hemocytometer. After standing to liquefy, the sample was mixed thoroughly and diluted 1:100 in a white cell pipet with 5% bicarbonate, 1% formalin in water. The diluted sample was introduced into a counting chamber. The total sperm count in 4 squares of 1 mm^2 each was determined and multiplied by 2.5×10^5 to calculate the number of sperm per ml of semen (Alexander, 1982).

Staining procedures. Sperm morphology was analyzed on Papanicolaou stained smears (Lillie, 1965). Air-dried slides were fixed in ethanol and stained with hematoxalin. They were then destained with 0.5% HCl and refixed in ethanol. They were then stained with Orange 6 followed by Eosin 65 (Papanicolaou). For F-body analysis, slides were fixed in methanol then stained in 1 μg/ml quinacrine hydrochloride in 5% ethanol in water for 45–50 min. The slides were rinsed and cover slips mounted with McIlvanes buffer (Dean, 1979) pH 5.5.

Sperm morphology analysis. Papanicolaou stained sperm were examined by brightfield illumination at 1250-fold magnification and classified into morphological types. The classification and scoring logic described by Wyrobek et al. (1982) were used. Sperm were classified by shape; oval (normal), small, large, round, double head, narrow at base of head, narrow, pear shaped, irregular and ghost. Sizes were discriminated using an eyepiece reticle previously calibrated with a stage micrometer. Only sperm with tails were evaluated. Scoring consistency was controlled by the use of reference standard slides from 9 subjects. The reference slides were scored repeatedly by the scorer as well as by 2 other individuals. They were then randomly inserted among the study slides and scored with them. A total of 500 sperm were scored for each sample. Results were recorded by the number in each classification and the proportion of abnormal sperm (the sum of all classes except oval) was calculated as a summary statistic.

F-body frequency analysis. F-body frequency was determined by examination of quinacrine stained slides on a Leitz reflected fluorescence microscope using a narrow excitation band at 436 nm and a 470-nm barrier filter. The scoring criteria described by Kapp and Jacobson (1980) were used. Only oval-shaped normal sized cells were scored in which F-bodies were totally contained within the sperm head without distortion around the F-body. 500 sperm were examined for each sample and the proportion containing zero, one, or two F-bodies determined. To control for uniformity of staining intensity, the brightness of the stain was noted and the proportion of cells containing one F-body was considered. Preparations which were too bright or too dim or in which the frequency of one F-body was below 40% were decolorized, restained and re-evaluated.

Sperm morphology analysis in mice. The effects on sperm morphology of formalin (37% formaldehyde, 10% methanol in water), methanol and cyclophosphamide were determined in $B_6C_3F_1$ mice. Groups of 4-month-old male mice (Charles River Breeding Laboratory, Portage, MI) were treated by oral gavage with 5 consecutive daily doses of: formalin (100 mg/kg; 10 animals), methanol (1000 mg/kg; 10 animals), cyclophosphamide (100 mg/kg; 5 animals), or distilled water (5 animals). The animals were maintained on standard laboratory feed and water ad libitum for 5 weeks after treatment. They were then sacrificed and the cauda epididymides dissected and flushed to recover the spermatozoa. The sperm were fixed, mounted on slides, stained with eosin Y and examined microscopically. Sperm were classified as normal, lacking hook, banana-like, amorphous or two-tailed (Wyrobek and Bruce, 1978). A total of 500 sperm were evaluated per animal and the proportion of abnormal sperm calculated as a summary statistic.

Sperm parameters were evaluated using the mean value for each parameter for all of the samples collected from each subject. Pearson correlation coefficients were calculated between sperm parameters and other parameters for which information on exposure level was available (e.g. num-

ber of cigarettes smoked per day). Incidental exposures for which dose data was not available were evaluated by analysis of variance. The effects of exposure and non-exposure for each factor were compared by Student's *t*-test. Parameters were evaluated in their original form (viz. sperm count, proportion of 0, 1, 2 F-bodies, proportion with abnormal morphology). Sperm count was evaluated using a transformed variable equal to the log (sperm count) and proportion of 2F-body bearing sperm was evaluated using a transformed variable equal to arcsin $\sqrt{2F}$. Results were evaluated with both the original and transformed variables. Analysis of sperm morphology in the mouse was performed using non-parametric tests to determine the significance of differences among all treatment groups (Kruskal–Wallis test) and between groups (Mann–Whitney *U* test). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) (Nie et al., 1975). The effect of time on variation was evaluated using the analysis of variance with repeated measures procedure of BMDP79 (Dixon, 1979).

Results

Population description. The study results are based on 11 pairs of individuals. Of the 11 exposed individuals, 9 spent all or a major portion of their time on the Autopsy Service while 2 were only present for gross pathology conferences twice a week. Of the 9 full-time employees, 4 were residents and 5 were technicians. 3 residents and 1 technician worked for 6 months on the Autopsy service beginning 2 months prior to the first sample collection and ending 1 month prior to the last sample collection. 1 resident worked 1 month on the service. Samples were collected at 1, 7 and 9 months after onset of exposure. The descriptive characteristics of the members of the exposed and control groups are presented in Table 1. The purpose of matching subjects in pairs was to obtain two populations as similar as possible in characteristics such as age, sex and lifestyle so that results could be evaluated on a population basis. There was no intent to evaluate results on a paired case-control basis.

Formaldehyde exposure. Formaldehyde exposure levels were determined in the Autopsy Service area using the chromotropic acid method (NIOSH, 1977). A detailed report of the results of that survey has been published (Coldiron et al., 1983). Both area and personal breathing zone samples were collected. The exposure experience could be best characterized as episodic with levels reaching a maximum of 5.8 ppm for certain specific activities and a minimum of 0.1 ppm (detection limit) for time spent outside of the immediate

TABLE 1

DESCRIPTION OF POPULATION CHARACTERISTICS

Characteristic	Group	
	Exposed	Control
Number	11	11
Age [a] < 30	7	9
< 40	3	1
< 50	0	0
> 50	1	1
Tobacco use		
none	9	8
< 10/day	2	3
Alcohol use		
none	1	1
< 1/day	3	5
1–4/day	7	5
Coffee use		
none	1	3
1–4/day	7	6
> 4/day	3	2
Marijuana use		
no	9	9
yes	2	2
Medications		
no	4	4
yes	7	7
Exposures [b]		
none	6	7
solvents	4	3
pesticides	0	2
diagnostic radiation	5	0
Viral infection		
no	8	7
yes	3	4

[a] Mean age difference of pairs: 4.1 ± 3.1 years.

[b] 1 control subject exposed to solvents and pesticides.
3 exposed subjects exposed to solvents and X-ray.

work area. Time weighted average exposures were estimated to be between 0.61 and 1.32 ppm with little difference between pathologists, residents and technicians. The least exposed individuals are estimated to have received a weekly exposure of about 3 ppm · h by attending gross autopsy conferences. Full-time employees received an exposure of 40 ppm · h or more.

Sample collection. 3 samples were obtained from each individual at 2–3 month intervals. A few samples were lost to analysis for technical reasons as shown in Table 2. The reported average prior continence for each group was 3.5 ± 3.9 days for the exposed group and 3.0 ± 2.1 days for the non-exposed group.

Participants in the study were tested 3 times at intervals longer than the 74-day duration of spermatogenesis (Heller and Clermont, 1964). For all 3 tests, an evaluation was made to determine whether a consistent trend was observed in changes of test values across time. No such changes were observed in either group; consequently, the 3 values were averaged and used for subsequent analysis.

Sperm count. Individual results of the sperm count analysis are presented in Table 2. Mean counts for individuals ranged from 5.3 to 153.3×10^6/cc in the exposed group and 2.7 to 258.5×10^6/cc in the control group. Although the group means differ by 24.5×10^6, the difference is not statistically significant.

TABLE 2

SUMMARY OF SPERM DATA BY STUDY SUBJECT

Subject No.	*n* samples	Sperm count [a]		% abnormal		% 1F-body		% 2F-body	
		Mean [b]	S.D.	Mean [b]	S.D.	Mean [b]	S.D.	Mean [b]	S.D.
Exposed group									
1	3	102.3	125.7	43.9	13.9	44.3	– [c]	0.90	– [c]
2	2	124.0	– [c]	31.9	0.1	51.0	– [c]	0.90	– [c]
3	2	10.0	– [d]	51.0	7.1	43.0	– [d]	1.40	– [d]
4	3	31.7	20.8	40.5	2.3	44.3	2.7	2.00	0.4
5	3	8.0	4.4	64.6	5.2	50.0	– [c]	2.20	– [c]
6	3	153.3	70.1	27.1	3.8	47.1	3.5	0.7	0.2
7	3	92.7	67.7	39.3	0.8	44.1	0.5	2.9	0.5
8	3	47.0	49.2	45.5	9.4	44.6	1.4	1.5	0.3
9	3	69.0	29.5	48.5	6.5	52.7	1.7	0.9	0.1
10	3	5.3	0.6	68.6	3.4	50.5	4.0	2.9	0.6
11	3	48.7	3.2	29.2	7.0	27.4	– [c]	0.7	– [c]
Total	31	62.9	49.9	44.5	13.4	45.4	6.9	1.6	0.8
Control group									
1	3	73.7	42.6	50.5	3.9	45.2	3.8	1.7	0.3
2	3	2.7	0.6	58.6	9.2	42.0	3.5	0.9	0.1
3	3	150.0	65.4	28.7	1.7	43.7	5.6	1.9	0.9
4	3	102.7	84.8	51.7	3.6	42.9	1.2	1.3	0.5
5	3	76.0	87.7	66.8	3.8	44.4	4.7	1.9	0.1
6	3	20.3	12.7	53.5	4.0	46.2	2.8	2.3	0.2
7	3	22.8	11.4	62.8	6.0	43.3	3.1	3.5	1.0
8	3	145.7	54.8	35.6	6.2	45.4	4.7	1.4	0.4
9	3	258.5	– [c]	69.2	0.5	45.6	0.6	0.8	0
10	3	30.5	– [c]	79.3	7.8	48.7	3.4	1.2	0.2
11	3	78.3	48.6	31.5	1.9	47.3	3.3	0.9	0.1
Total	33	87.4	75.0	53.5	16.2	45.0	2.0	1.6	0.8

[a] Sperm count in millions/cc of semen.
[b] Mean values are based on 3 samples per individual except where indicated.
[c] Based on 2 samples.
[d] Based on 1 sample.

F-body frequency. Individual and group statistics for F-body frequency are also presented in Table 2. Frequencies of sperm containing 1F- or 2F-bodies are very similar in the exposed and control groups, and, again, no significant effect can be ascribed to formaldehyde exposure. Both the consistency and level of the frequency of 1F-body suggest that staining procedures were uniform throughout the evaluation processes and that variation in staining was not a significant problem in scoring.

Sperm morphology. The individual and group statistics for the percentage of abnormally shaped sperm are presented in Table 2. The percentage of abnormal forms were actually greater in the control group than in the exposed group, however, the difference was not statistically significant ($p > 0.17$).

Results of all 3 tests from the 5 residents and 1 technician who rotated on and off the service during the sampling period were examined for trends associated with changes in their exposure. No trends were observed.

Mouse sperm morphology. In order to determine the potential of formaldehyde to induce sperm abnormalities, a study was performed in $B_6C_3F_1$ mice. The mice were exposed to formaldehyde at 100 mg/kg/day × 5 days which was much higher than the exposure encountered by the human study group. The same treatment regimen at 250 and 500 mg/kg was lethal to all animals (10/10) treated. Also intraperitoneal injection of 5 daily doses of 100 mg/kg was lethal to all animals treated. The results of the study are presented in Table 3. Overall results indicate a small but non-significant increase in the proportion of abnormal forms in animals treated with formalin. Treatment with methanol at a dose 10 times that present in the formalin produced a similar but non-significant increase in abnormality. In contrast, a single dose of 100 mg/kg cyclophosphamide produced a more than 5-fold increase in morphological abnormality compared to the negative control. Significant differences among treatments were observed for individual sperm shape classes as indicated in Table 3. The proportion of banana-type sperm was elevated in formalin and methanol treated animals. The biological significance of these changes is unknown. While conclusions must be made cautiously from a single dose experiment, treatment at near lethal levels was not found to produce morphological abnormalities in mouse sperm.

Other associations. The sperm test results were evaluated for associations with age or lifestyle characteristics used in matching. Abnormal morphology and 2F-body frequency were inversely correlated with log sperm count in the exposed group ($r = -0.88$, $p = 0.001$; $r = -0.60$, $p = 0.03$, respectively) but not the non-exposed group. The use of marijuana was associated with reduced sperm count ($p < 0.02$) which has been previously

TABLE 3

SPERM MORPHOLOGY IN $B_6C_3F_1$ MICE EXPOSED TO FORMALIN

	Dose	Number of animals	Abnormal morphologies [a]			2 Tails	% ± S.D.
			Lacks hook	Banana	Amorphous		
Water	–	5	1.5	0	4.2	0.2	1.12 ± 0.39
Formalin	100 mg/kg × 5	7	2.3	2.6 [b]	2.3	0.28	1.49 ± 0.90
Methanol	1 g/kg × 5	10	3.3	4.0 [b]	2.7	0.1	1.86 ± 0.91
Cytoxan	100/kg a dose	5	6.4 [b]	0.6 [b]	21.2 [b]	1.0	5.84 ± 1.94 [b]

[a] Number of abnormalities per animal (500 sperm scored per animal).

[b] Significantly higher ($p < 0.01$) than water control for morphological type by Mann–Whitney U test (Hull and Nie, 1979).

reported (Hembree et al., 1979; Takahashi et al., 1981; Huang et al., 1979). The use of medications was significantly associated ($p < 0.004$) with abnormal morphology in the control group only. No significant associations were seen with the other lifestyle characteristics evaluated.

Discussion

We have reported the results of the analysis of endpoints in sperm in workers occupationally exposed to formaldehyde on an autopsy service. The 3 sperm variables evaluated were count, distribution of morphological types and frequency of sperm containing zero, one or two F-bodies. All 3 endpoints have been used as indirect indicators of exposure to genetically toxic agents. We have previously discussed the relevance of these tests for inclusion in a battery of tests for genetic monitoring (Ward et al., 1983). The genetic basis for these tests as well as the record of their use is discussed in detail in the recent reports of the Environmental Protection Agency Gene-Tox Program for both man (Wyrobek et al., 1983a) and animals (Wyrobek et al., 1983b).

Analysis of the data for the formaldehyde exposed and non-exposed populations did not reveal significant differences between them in any of the 3 parameters. In addition, exposure of $B_6C_3F_1$ mice to concentrations of formaldehyde approaching lethal levels did not produce abnormalities in sperm morphology. Although formaldehyde is genotoxic in several systems as previously noted, the lack of apparent activity might be accounted for in several ways. Genetic damage might be rapidly repaired as suggested by the rapid repair of DNA–protein crosslinks in mammalian cells (Ross and Shipley, 1980). Formaldehyde is readily bound to tissues (Heck et al., 1983) and rapidly metabolized (IARC, 1983) reducing the likelihood that significant concentrations would reach the testes. However, the possibility that toxic cross-linking products might be formed remains a concern. The genotoxic activity of such products has been suggested by studies with human cells (Feldman and Balabanova, 1978), Drosophila (Auerbach et al., 1977) and mice (Fontignie-Houbrechts et al., 1982). However, if such products were formed in the formaldehyde-exposed humans, they produced no detectable effect on sperm. Clearly, it is also possible that effects were not seen because the study lacked sufficient statistical power to detect effects at this exposure level in a small population.

No significant differences between the exposure groups were detected for these sperm parameters, nor was any pattern of variation among replicate samples observed over time. Given the sizes of the exposure groups and the standard errors in the control group, the following differences between exposed and non-exposed groups would have been detected at a significance of $p < 0.05$ and a power of 80% (Brown and Herson, 1981): sperm count of 74×10^6 sperm/ml; 2F-body frequency of 0.8%; abnormal morphology of 16.1%. Clearly, smaller differences in true means could exist between the exposure groups without being detected. A larger population would have increased the sensitivity of the study, however, all but one or two members of the autopsy service staff participated in this study. At the minimum, this study demonstrates that formaldehyde exposure did not produce moderate to severe effects in male germ cells in this population. It remains possible that minor effects might have occurred which could not be detected in this study.

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